# Properties of [$Ca^{2+}$ + $Mg^{2+}$]-Adenosine Triphosphatases in the Golgi Apparatus and Microsomes of the Lactating Mammary Glands of Cows[1]

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## ABSTRACT

The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activity of bovine lactating mammary gland is associated with membranes. This study compares the ATPase activity in microsomal membranes to that of the Golgi apparatus. The enzyme activity in both fractions hydrolyzed $Ca^{2+}$-ATP and $Mg^{2+}$-ATP. The ATPase activities were inhibited by *p*-chloromercuribenzoate, indicating the involvement of a sulfhydryl group for activity. Although calmodulin had no effect on the ATPase activities of the two fractions, calmodulin antagonists (chlorpromazine, fluphenazine, and trifluoperazine) were inhibitory. Strong inhibitors of the ATPase activities were vanadate, dicyclohexylcarbodiimide, $La^{3+}$, and $Zn^{2+}$. There were some differences in the activities from two membrane fractions. Although both fractions could hydrolyze all of the triphosphonucleotides, cytidine-5′-triphosphate and uridine-5′-triphosphate were poor substrates for the Golgi enzyme. Detergents diminished the activity of the microsomal enzyme to a much greater extent than the ATPase of the Golgi apparatus. Thus, the intact membrane may be more critical to microsomal activity. The role of these enzymes in $Ca^{2+}$ accumulation in milk is discussed.

**(Key words**: enzymes, membranes, milk, secretion)

[1]Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

**Abbreviation key**: **CTP** = cytidine-5′-triphosphate; **DCCD** = dicyclohexylcarbodiimide; **DTT** = dithiothreitol; **EGTA** = ethylene glycol bis(β-aminoethyl ether)-N,N,N′N′-tetraacetic acid; **GTP** = guanosine-5′-triphosphate; **MOPS** = 3-(N-morpholino)propane sulfonic acid; **NEM** = N-ethylmaleimide; **PCMB** = *p*-chloromercuribenzoate; **TPPase** = thiamine pyrophosphatase; **UTP** = uridine-5′-triphosphate.

## INTRODUCTION

During lactation, the secretory cells of the mammary gland transport large amounts of calcium from blood into milk. In a high producing dairy cow, the total calcium content of blood is 2.5 to 3 m*M*, whereas milk contains approximately 31 m*M* (7). Thus, a 10-fold increase in the concentration of calcium is achieved.

In bovine milk, two-thirds of the calcium is bound to casein; most of the rest is bound to citrate, and small amounts are present as free ions (7). The association of calcium with casein results in the formation of a colloidal complex—the casein micelle. The presence of $Ca^{2+}$ and the phosphorylation of certain serine residues in casein are required for micelle formation (6). The nascent casein molecules are phosphorylated by a casein kinase located in the Golgi apparatus (2). Calcium then forms bonds with the phosphate groups of casein, resulting in colloidal micelles (3). Evidence of micelle formation in the Golgi apparatus can also be seen in studies using electron microscopy (6, 20).

It is thought that a calcium pump is involved in the accumulation of $Ca^{2+}$ in the Golgi apparatus. Therefore, most of the studies

of calcium transport in the lactating mammary gland have been concerned with the $Ca^{2+}$-ATPases of the Golgi apparatus. Baumrucker and Keenan (1) described an ATPase in the Golgi-enriched fractions from bovine mammary gland. The ATPase activity required $Mg^{2+}$ and was stimulated by $Ca^{2+}$ and KCl. This enzyme was also found in microsomes and in the fat globule membranes of cream. Golgi-enriched membranes from lactating murine mammary tissue exhibited $Ca^{2+}$-stimulated ATPase activity in the presence of free $Mg^{2+}$ and $Mg^{2+}$-ATPase (18). In addition to ATPase activity, mammary Golgi membranes have the ability to accumulate calcium. Evidence for this calcium pump activity has been obtained from studies on cows (1), mice (18), and rats (17, 19).

This study deals with the subcellular distribution of divalent cation-stimulated ATPase activity in lactating mammary glands of cows. The ATPase activities in microsomal and Golgi apparatus membranes are investigated to clarify their roles in $Ca^{2+}$ transport through the endomembrane system of the lactating mammary gland and to understand better the mechanism involved in the transport of $Ca^{2+}$ into milk.

## MATERIALS AND METHODS

### Materials

Mammary glands were obtained from cows in full lactation through the cooperation of John E. Keyes (Beltsville Agricultural Center, Beltsville, MD). Following slaughter, the glands were trimmed of extraneous fat, cut into pieces (approximately 200 g), and stored at –80°C.

Dithiothreitol (**DTT**), *p*-chloromercuribenzoic acid (**PCMB**), 1-levamisole, sodium orthovanadate, 2-mercaptoethanol, calmodulin, ouabain, oligomycin (mixture of A, B, and C), malachite green hydrochloride, trifluoperazine, the Tris salt of ATP, chlorpromazine hydrochloride, quercetin, sodium salts of the nucleic acids, and 3-(N-morpholino)propane sulfonic acid (**MOPS**) buffer were obtained from Sigma Chemical Company (St. Louis, MO). The protein assay reagent and Triton X-100 were purchased from Pierce Chemical Company (Rockford, IL), and fluphenazine was from Squibb Company (Princeton, NJ). Dicyclohexylcarbodiimide (**DCCD**), *n*-octyl-β-D-glucopyranoside, and ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (**EGTA**) were obtained from Calbiochem (San Diego, CA).

### Tissue Fractionation

Golgi apparatus was isolated from frozen mammary gland by the method of Morré (11) as modified by Bingham et al. (2). Mammary glands (100 g) were minced and suspended in 3 volumes of homogenization buffer, which consisted of Medium A (37.5 m*M* Tris-maleate buffer, pH = 6.5, 1 m*M* EDTA, 25 m*M* $MgCl_2$, and .1 m*M* DTT) containing .5 *M* sucrose and 1% dextran. The mixture was homogenized for 1 min (medium speed) with a Polytron 10 ST (Brinkman, Westbury, NY). The homogenate was filtered first through one and then through two layers of cheesecloth to remove unbroken cells and connective tissue. The preparation was then centrifuged for 15 min at 4000 × *g* in a swinging bucket rotor. The pellet was washed once with the homogenization buffer and resedimented. The supernatant solution was carefully removed so that the pellet was not disturbed. The yellow-brown, friable, top one-third portion of the pellet contains the Golgi apparatus. By gently swirling the tube with homogenization buffer, it was possible for us to extract the fraction containing the Golgi membranes from the pellet. The suspension of the Golgi apparatus in homogenization buffer was layered over 1.5 volumes of Medium A containing 1.25 *M* sucrose and 1% dextran and was then centrifuged at 100,000 × *g* for 30 min. The Golgi fraction, concentrated at the interface of the two layers, was removed with a Pasteur pipet, diluted with homogenization buffer, and pelleted at 4000 × *g* for 15 min. The Golgi membranes were stored in the homogenization buffer at –80°C.

For subsequent fractionations, the supernatants and pellets remaining after the recovery of the Golgi apparatus fraction were combined, diluted with Medium A to give a final sucrose concentration of .25 *M*, and homogenized gently with a Teflon®-glass homogenizer (2). Nuclei, mitochondria, microsomes, and cytoplasm were prepared by differential centrifugation as described by Schneider (15). All

pellets were suspended in Medium A, sonicated, and stored at –80°C.

### Protein Assay

Protein was determined with the bicinchoninic acid protein assay reagent (Pierce Chemical Company) at 25°C and standard protocol. Bovine serum albumin was used as the standard.

### Enzyme Assays

The NADPH-cytochrome *c* reductase, lactose synthetase, succinic dehydrogenase, and thiamine pyrophosphatase (**TPPase**) were determined by the methods of Masters et al. (8), Pennington (14), Palmiter (12), and Farrell et al. (5), respectively. The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities in subcellular fractions were determined at pH 7.4 in a .5-ml reaction mixture containing 430 $\mu M$ $CaCl_2$, 109 $\mu M$ $MgCl_2$, 504 $\mu M$ EGTA, .05 *M* MOPS, and 155 $\mu M$ ATP. The concentration of reactants, calculated by the procedure of Watters (18), contained 20 $\mu M$ free $Ca^{2+}$, 40 $\mu M$ free $Mg^{2+}$, 60 $\mu M$ $Mg^{2+}$-ATP, and 400 $\mu M$ $Ca^{2+}$-EGTA. The reaction was initiated by the addition of 30 to 50 $\mu$g/ml of ATPase preparation and incubated for 15 min at 37°C. The inorganic phosphorus was determined by the addition of a malachite green-molybdate-polyvinyl alcohol reagent, as described by Chan et al. (4). The green solution was read in a spectrophotometer at 630 nmr. One unit of ATPase activity was defined as the amount of enzyme that produced 1 nmol of P in 1 min at 37°C. Under the assay conditions used, the ATPase increased linearly with the amount of added enzyme and with incubation time. Results are expressed as averages and standard deviations.

For studies on effectors and substrate specificity, ATPase activities were determined in an assay mixture containing 700 $\mu M$ $CaCl_2$, 550 $\mu M$ $MgCl_2$, 420 $\mu M$ EGTA, .05 *M* MOPS, and 710 $\mu M$ ATP to take into account the optimal condition for these fractions.

The effect of $Ca^{2+}$ on ATPase activity was determined in the presence of .4 m$M$ $Ca^{2+}$-ATP and .4 m$M$ $Ca^{2+}$-EGTA. The $Ca^{2+}$ (free) was varied from 4 to 200 $\mu M$. Total concentrations of the solutions in the assay mixtures were determined by the following equations.

$$\text{Total } Ca^{2+} = Ca^{2+} \text{ (free)} + Ca^{2+}\text{-ATP} + Ca^{2+}\text{-EGTA}.$$

$$\text{Total ATP} = Ca^{2+}\text{-ATP} + \text{ATP (free)}.$$

$$\text{Total EGTA} = Ca^{2+}\text{-EGTA} + \text{EGTA (free)}.$$

The dissociation constants for $Ca^{2+}$-ATP and $Ca^{2+}$-EGTA are .1122 and .0048 m$M$, respectively (18). These values were used to determine the concentrations of ATP (free) and EGTA (free) in the equations just given. The membrane preparations used in these experiments were dialyzed for 48 h against 30 m$M$ MOPS buffer, pH 7.4.

The effect of $Mg^{2+}$ on ATPase activity was determined in a similar manner. Activity was measured in the presence of .1 m$M$ $Mg^{2+}$-ATP, .01 m$M$ $Mg^{2+}$-EGTA, and free $Mg^{2+}$ (1 to 200 $\mu M$). The dissociation constants for $Mg^{2+}$-ATP and $Mg^{2+}$-EGTA are .0537 and .4365 m$M$, respectively. Activities of ATPase were determined on .5 ml of reaction mixture as described.

TABLE 1. Subcellular distribution of [$Ca^{2+}$ + $Mg^{2+}$]-ATPase and marker enzymes in the lactating bovine mammary gland.[1]

| Subcellular fraction | Specific activity | | | Enzyme marker ratio | |
|---|---|---|---|---|---|
| | ATPase[2] | | TPPase[3] | | |
| | (nmol/min per mg of protein) | | | | |
| | $\bar{X}$ | SD | $\bar{X}$ | $\bar{X}$ | SD |
| Homogenate | 66.4 | 2.4 | 11.2 | 1.0 | |
| Nuclei | 84.1 | 3.7 | 13.3 | | |
| Mitochondria | 115.7 | 17.0 | 33.9 | 7.8[4] | .9 |
| Golgi | 125.1 | 12.0 | 58.2 | 9.7[5] | 2.9 |
| Microsomes | 89.5 | 16.0 | 23.6 | 5.5[6] | 1.5 |
| Cytosol | 10.0 | 2.0 | 16.4 | | |

[1]Enzymes in the subcellular organelles were assayed according to procedures in Materials and Methods.

[2]Mean of three preparations.

[3]Thiamine pyrophosphatase activity of subcellular fractions. Mean of two preparations.

[4]Succinic dehydrogenase; ratio of specific activity in mitrochondria to homogenate. Mean of three preparations.

[5]Lactose synthetase; ratio of specific activity in Golgi apparatus to homogenate. Mean of eight preparations.

[6]Cytochrome *c* reductase; ratio of specific activity in microsomes to homogenate. Mean of five preparations.

## RESULTS

### Subcellular Distribution of ATPase

Subcellular fractions of lactating bovine mammary gland were isolated by differential centrifugation and assayed for ATPase activity (Table 1). Lactose synthetase, succinate dehydrogenase, and NADPH:cytochrome *c* reductase were used as marker enzymes for Golgi apparatus, mitochondria, and microsomes, respectively. Lactose synthetase was enriched 10-fold in . the Golgi, whereas NADPH:cytochrome *c* reductase increased over 5-fold in the microsomes. The succinate dehydrogenase in the mitochondria was eight times that found in the homogenate. Relatively little cross-contamination (~10%) occurred, as determined by electron microscopy as previously described (16). The distribution of TPPase, a marker enzyme for the *trans*-Golgi, is shown in detail. There appears to be some generalized phosphatase activity in the bovine mammary gland because the cytosolic fraction exhibits a specific activity greater than the homogenate. In contrast, TPPase activity in the Golgi apparatus was five times that in the homogenate; unlike this marker enzyme, the specific activity of the ATPase showed relatively little variation among the membrane fractions. The cytosolic fraction, however, showed decreased ATPase activity, indicating that it is predominantly membrane-associated. Specific activities (nmol/min per mg) varied from 84 in the nuclei to 125 in the Golgi apparatus. The membrane fractions all showed increased specific activities over the homogenate. However, the specific activity of the cystolic ATPase was only 12% of that in the homogenate.

### Effect of Divalent Ions on ATPase Activity

The activities of the ATPases in the microsomes and the Golgi apparatus were tested in the presence of divalent metal ions. In both membrane preparations, $Ca^{2+}$ or $Mg^{2+}$ were required for activity (Figures 1 and 2); very little activity occurred in the absence of these cations. In the presence of $Ca^{2+}$, the ATPase activities increased with the concentration of $Ca^{2+}$ (Figure 1). When the effect of $Mg^{2+}$ was tested, both membrane ATPase activities ob-

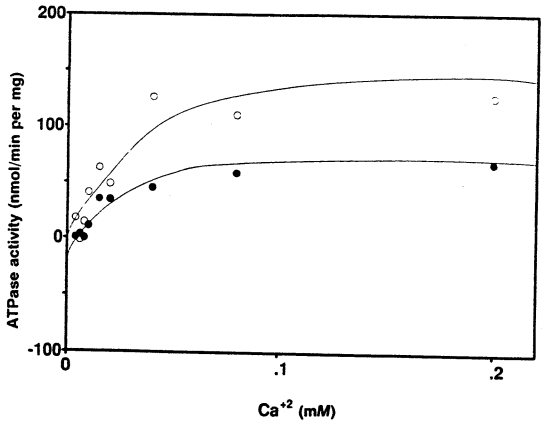


Figure 1. The ATPase activity plotted against calculated free $Ca^{2+}$ concentration. Curves show microsomes (●) and Golgi apparatus (○). Assays using dialyzed membrane preparations are described in Materials and Methods.

tained maximum activity between .02 and .03 m*M* $Mg^{2+}$; the activity declined at higher $Mg^{2+}$ concentrations.

### Role of Sulfhydryl Groups

Sulfhydryl-protecting agents (cysteine, DTT, and 2-mercaptoethanol) did not stimulate

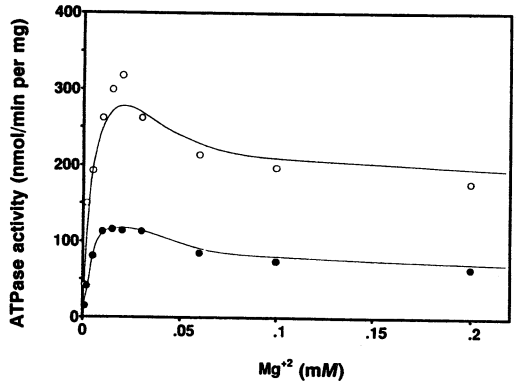


Figure 2. The ATPase activity plotted against calculated free $Mg^{2+}$ concentration. Curves show microsomes (●) and Golgi apparatus (○). Assays using dialyzed membrane preparations are described in Materials and Methods.

TABLE 2. Effect of sulfhydryl-protecting and sulfhydryl-blocking agents on [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activity in the lactating bovine mammary gland.[1]

| Effector | Activity | | | |
|---|---|---|---|---|
| | Golgi apparatus | | Microsomes | |
| | (%) | | | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD |
| None (control) | 100 | | 100 | |
| Cysteine | 102 | 9 | 98 | 25 |
| Dithiothreitol | 96 | 15 | 97 | 15 |
| 2-Mercaptoethanol | 85 | 8 | 102 | 9 |
| PCMB | 47 | 11 | 55 | 1 |
| PCMB + cysteine | 105 | 12 | 99 | 2 |
| PCMB + dithiothreitol | 91 | 6 | 100 | 3 |
| PCMB + 2-mercaptoethanol | 88 | 13 | 87 | 8 |
| NEM | 94 | 5 | 94 | 8 |
| NEM + 2-mercaptoethanol | 90 | 21 | 88 | 9 |

[1]The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities were assayed according to procedures in Materials and Methods. Results are expressed as percentages of the control and are mean values obtained from three cows. *p*-Chloromercuribenzoate (PCMB) and N-ethylmaleimide (NEM) were .2 m*M*. Sulfhydryl-protecting agents (cysteine, dithiothreitol, and 2-mercaptoethanol) were .4 m*M*.

ATPase activity in the Golgi or microsomal membranes, although PCMB, a sulfhydryl-blocking agent, inhibited both enzyme activities (Table 2). Addition of the sulfhydryl-protecting agents reversed the inhibition. N-Ethylmaleimide (NEM), another sulfhydryl-blocking agent, had little effect on the ATPase activities. Apolar organomercurials, such as PCMB, are much more effective in their reaction with the hydrophobic sulfhydryl groups than hydrophilic reagents, such as NEM (10).

### Effect of Other Inhibitors

Table 3 summarizes the effect of various ATPase inhibitors. Ouabain and NEM (see Table 2), which are inhibitors of Na-K-ATPases, had little effect on the ATPase activities of Golgi and microsomal membranes. Oligomycin, an inhibitor of mitochondrial ATPase, only slightly affected the ATPase activities of the two membrane preparations. Sodium vanadate, a structural analogue of phosphate, strongly inhibited the ATPase activities. Vanadate is a potent inhibitor of the P-type of ATPase (13). The ATPase was slightly inhibited in the presence of molybdate, which is a general inhibitor of phosphatases.

### Effect of Metal Ions

Table 4 summarizes the effect of metal ions on Golgi and microsomal ATPase activities. The strongest inhibition of both fractions was obtained in the presence of $Zn^{2+}$ and $La^{2+}$. Golgi ATPase activity, but not microsomal ATPase activity, was inhibited by $Fe^{2+}$ and $Mn^{2+}$.

TABLE 3. Effect of various inhibitors on [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activity in the lactating bovine mammary gland.[1]

| Effector | Activity | | | |
|---|---|---|---|---|
| | Golgi apparatus | | Microsomes | |
| | (%) | | | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD |
| None (control) | 100 | | 100 | |
| Oligomycin (20 μg/ml) | 76 | 9 | 83 | 7 |
| Sodium vanadate (.5 m*M*) | 38 | 7 | 30 | 1 |
| Quercetin (1 m*M*) | 82 | 3 | 94 | 0 |
| DCCD (.1%) | 53 | 2 | 58 | 11 |
| Ammonium molybdate (1 m*M*) | 94 | 1 | 89 | 2 |
| Ouabain (5 m*M*) | 106 | 9 | 111 | 3 |

[1]The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities were assayed according to procedures in Materials and Methods. The reagents were included in the assays at the indicated concentrations. Results are expressed as percentages of the control and are mean values obtained from three cows. DCCD = Dicyclohexylcarbodiimide.

TABLE 4. Effect of metal ions on [$Ca^{2+}$ + $Mg^{2+}$]-ATPase in the lactating bovine mammary gland.[1]

| Metal ion | Activity | | | |
|---|---|---|---|---|
| | Golgi apparatus | | Microsomes | |
| | (%) | | | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD |
| $MgCl_2$ (control) | 100 | | 100 | |
| KCl (100 m*M*) | 104 | 2 | 98 | 3 |
| NaCl (100 m*M*) | 81 | 6 | 80 | 3 |
| $CuSO_4$ (.01 m*M*) | 91 | 1 | 117 | 3 |
| $FeSO_4$ (1 m*M*) | 74 | 1 | 97 | 1 |
| $MnSO_4$ (1 m*M*) | 76 | 1 | 92 | 2 |
| $ZnCl_2$ (.1 m*M*) | 31 | 4 | 43 | 3 |
| $LaCl_3$ (.1 m*M*) | 11 | 3 | 58 | 4 |

[1]The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities were measured according to the procedures in Materials and Methods. The indicated metal ions were used in the assay. Results are expressed as percentages of the control and are mean values obtained from three cows.

### Substrate Specificity

Table 5 summarizes the reactivities toward various nucleotide triphosphates. The microsomal ATPase used all the substrates well, but the Golgi ATPase showed diminished activity toward cytidine-5′-triphosphate (**CTP**), guanosine-5′-triphosphate (**GTP**), and uridine-5′-triphosphate (**UTP**).

### Effect of Detergents on Activity

The treatment of microsomes with detergents (Triton X-100 and *n*-octyl-β-D-glucopyranoside) greatly diminished the ATPase activity of the microsomal membranes (Table 6). The Golgi enzyme was less affected by treatment with detergents. The results suggest that the intact membrane may be important for the proper functioning of the microsomal ATPase.

### Effect of Calmodulin

The addition of calmodulin (10 μg/ml) failed to increase the ATPase activity (Table 7). However, possible calmodulin dependency was shown by using the calmodulin antagonists (fluphenazine, chlorpromazine, and trifluoperazine), which inhibited the ATPase activity.

TABLE 6. Effect of detergents on [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activity from the lactating bovine mammary gland.

| Detergent | Activity | | | |
|---|---|---|---|---|
| | Golgi apparatus | | Microsomes | |
| | (%) | | | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD |
| None (control) | 100 | | 100 | |
| Triton X-100 | | | | |
| 1% | 76 | 0 | 26 | 1 |
| .5% | 97 | 1 | 65 | 1 |
| .1% | 98 | 1 | 72 | 1 |
| *n*-Octyl glucoside | | | | |
| .5% | 71 | 1 | 27 | 1 |
| .1% | 84 | 1 | 24 | 1 |

The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities were assayed according to procedures in Materials and Methods. The membrane preparations were homogenized with the detergents at the indicated concentrations. Results are expressed as percentages of the control and are mean values obtained from three cows.

## DISCUSSION

Although large amounts of calcium are secreted by mammary cells during lactation, very little is known about the translocation of $Ca^{2+}$ into milk. Previous research on mammary tissue focused on the Golgi apparatus. This research found ATPase activities in all of the membrane fractions (Table 1). The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities of the microsomal

TABLE 5. Hydrolysis of nucleotides by [$Ca^{2+}$ + $Mg^{2+}$]-ATPase from the lactating bovine mammary gland.[1]

| Nucleotide triphosphate | Activity | | | |
|---|---|---|---|---|
| | Golgi apparatus | | Microsomes | |
| | (%) | | | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD |
| ATP (control) | 100 | | 100 | |
| CTP | 44 | 9 | 82 | 9 |
| GTP | 83 | 6 | 114 | 1 |
| ITP | 108 | 5 | 131 | 4 |
| UTP | 59 | 3 | 103 | 2 |

[1]The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities were assayed according to procedures in Materials and Methods. Results are expressed as percentages of the control and are mean values obtained from three cows. The nucleotides that replaced ATP in the reaction mixture were cytidine-5′-triphosphate (CTP), guanosine-5′-triphosphate (GTP), inosine-5′-triphosphate, (ITP), and uridine-5′-triphosphate (UTP).

TABLE 7. Effect of calmodulin and calmodulin-inhibiting reagents on [$Ca^{2+}$ + $Mg^{2+}$]ATPase activity from the lactating bovine mammary gland.

| Effector | Activity | |
|---|---|---|
| | Golgi apparatus | Microsomes |
| | (%) | |
| None (control) | 100 | 100 |
| Calmodulin (10 μg/ml) | 99 | 99 |
| Fluphenazine (.1 m*M*) | 46 | 50 |
| Chlorpromazine (.1 m*M*) | 45 | 67 |
| Trifluoperazine (.1 m*M*) | 55 | 67 |

The [$Ca^{2+}$ + $Mg^{2+}$]-ATPase activities were assayed according to procedures in Materials and Methods. Results are expressed as percentages of the control. Membrane samples were prepared in the reaction mixture with or without the calmodulin or calmodulin antagonists and incubated for 2 min. Then ATP was added, and the mixture was incubated for 10 min.

membranes and the Golgi apparatus were examined in detail because these membranes probably function in the secretory process.

The ATPases involved in ion translocation have been classified into three groups (P, F, and V) by Pedersen and Carafoli (13). The predominant mammary ATPases would be classified as the P type because vanadate was a more potent inhibitor than NEM and oligomycin, which are inhibitors of V- and F-type ATPases (13). The lack of inhibition by ouabain and NEM suggests that the enzymes differ from the plasma membrane [$Na^+$ + $K^+$]-ATPases. Inhibition by quercetin (1 m*M*) is slight. Quercetin (50 $\mu M$) did not inhibit murine Golgi $Ca^{2+}$-ATPase, although the enzymes from sarcoplasmic-reticulum and red cell plasma membranes were completely inhibited (18). In addition, the mammary ATPase activity, as that of most $Ca^{2+}$-ATPases, was inhibited by $La^{3+}$.

Calmodulin did not enhance ATPase activities. However, the calmodulin antagonists (fluphenazine, chlorpromazine, and trifluoperazine) strongly inhibited the microsomal and Golgi ATPases. The discrepancy between the ineffectiveness of added calmodulin and inhibition by added calmodulin antagonists may be accounted for by the presence of a calmodulin-binding protein (9).

Some differences existed between the two membrane ATPases. The metal salts ($FeSO_4$ and $MnSO_4$) inhibited the Golgi apparatus, but not the microsomal membranes. However, both enzymes were inhibited by $ZnCl_2$ and $LaCl_3$. The substrate specificity (see Table 5) differed. The Golgi enzyme hydrolyzed ATP and inosine-5′-triphosphate at a higher rate than CTP, GTP, and UTP. However, the microsomal membranes hydrolyzed all of the nucleotides at relatively high rates. The detergents, Triton X-100 and *n*-octyl-$\beta$-D-glucopyranoside, strongly inhibited the microsomal ATPase and had less effect on the Golgi ATPase activity. Whether these differences can be attributed to differences in the enzyme molecules or the membrane environment cannot be determined at the present time.

The translocation of calcium from blood to milk can be supported by the ATPase activities described in this report. The microsomal activity, which contains endoplasmic reticulum and plasma membrane, with a specific activity of 90 nmol of P/min per mg could support $Ca^{2+}$ transfer from the basement membrane to cytosol. The Golgi enzyme has a specific activity of 125 nmol of P/min per mg and could function to transfer $Ca^{2+}$ into secretory vesicles. Because the specific activity of casein kinase in a similarly prepared Golgi has been reported to be 3.1 nmol/min per mg (16), given the ratio of $Ca^{2+}$ to $PO_4$ as 1 for casein and assuming the ratio of 1:1 for ATP hydrolyzed to $Ca^{2+}$ carried, then the specific activity of the ATPase herein (125 nmol/min per mg) would adequately saturate newly phosphorylated casein molecules. The $Ca^{2+}$-enzyme may thus support milk protein secretion and is most likely not rate-limiting in the process.

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